of what has been possible in either nature or the laboratory. The safety of any proposed recombination will have to be weighed against its novelty and the possibility that it may never have occurred in the 3.5 billion years of evolutionary history.

References

1. Gibson, D.G., Glass, J.I., Lartigue, C., Noskov, V.N., Chuang, R.Y., Algire, M.A., Benders, G.A., Montague, M.G., Ma, L., Moodie, M.M., *et al.* (2010). Creation of a bacterial cell controlled by a chemically synthesized genome. Science *329*, 52–56.
2. Sanchez-Amat, A., Solano, F., and Lucas-Elio, P. (2010). Finding new enzymes from bacterial physiology: a successful approach illustrated by the detection of novel oxidases in *Marinomonas mediterranea*. Mar. Drugs *8*, 519–541.
3. Itaya, M., Tsuge, K., Koizumi, M., and Fujita, K. (2005). Combining two genomes in one cell: stable cloning of the *Synechocystis* PCC6803 genome in the *Bacillus subtilis* 168 genome. Proc. Natl. Acad. Sci. USA *102*, 15971–15976.
4. Lartigue, C., Glass, J.I., Alperovich, N., Pieper, R., Parmar, P.P., Hutchison, C.A., 3rd, Smith, H.O., and Venter, J.C. (2007). Genome transplantation in bacteria: changing one species to another. Science *317*, 632–638.
5. Sinoquet, C., Demey, S., and Braun, F. (2008). Large-scale computational and statistical analyses of high transcription potentialities in 32 prokaryotic genomes. Nucleic Acids Res. *36*, 3332–3340.
6. Francez-Charlot, A., Frunzke, J., Reichen, C., Ebneter, J.Z., Gourion, B., and Vorholt, J.A. (2009). Sigma factor mimicry involved in regulation of general stress response. Proc. Natl. Acad. Sci. USA *106*, 3467–3472.
7. Boucher, Y., Douady, C.J., Papke, R.T., Walsh, D.A., Boudreau, M.E., Nesbo, C.L., Case, R.J., and Doolittle, W.F. (2003). Lateral gene transfer and the origins of prokaryotic groups. Annu. Rev. Genet. *37*, 283–328.
8. Glass, J.I., Assad-Garcia, N., Alperovich, N., Yooseph, S., Lewis, M.R., Maruf, M., Hutchison, C.A., 3rd, Smith, H.O., and Venter, J.C. (2006). Essential genes of a minimal bacterium. Proc. Natl. Acad. Sci. USA *103*, 425–430.
9. Gogarten, J.P., Doolittle, W.F., and Lawrence, J.G. (2002). Prokaryotic evolution in light of gene transfer. Mol. Biol. Evol. *19*, 2226–2238.
10. Lawrence, J.G. (1997). Selfish operons and speciation by gene transfer. Trends Microbiol. *5*, 355–359.
11. Cohan, F.M., and Koeppel, A.F. (2008). The origins of ecological diversity in prokaryotes. Curr. Biol. *18*, R1024–R1034.
12. Koonin, E.V. (2007). The Biological Big Bang model for the major transitions in evolution. Biol. Direct *2*, 21.
13. Schimel, J., Balser, T.C., and Wallenstein, M. (2007). Microbial stress-response physiology and its implications for ecosystem function. Ecology *88*, 1386–1394.
14. Wu, D., Hugenholtz, P., Mavromatis, K., Pukall, R., Dalin, E., Ivanova, N.N., Kunin, V., Goodwin, L., Wu, M., Tindall, B.J., *et al.* (2009). A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. Nature *462*, 1056–1060.
15. Garfinkel, M.S., Endy, D., Epstein, G.L., and Friedman, R.M. (2007). Synthetic genomics — options for governance. Biosecur. Bioterror. *5*, 359–362.

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Bacterial Invasion: Entry through the Exocyst Door

Salmonella **entry into host cells involves rearrangements of actin and mobilization of membranes. Here we discuss new findings showing that *Salmonella* recruits the exocyst complex, which plays a role in vesicle secretion, to the site of invasion to promote its entry.**

Virginie Braun[1] and John H. Brumell[1,2,3,*]

Exocytosis is a fundamental cellular process by which a cell secretes proteins or lipids. It involves the tethering, docking and fusion of intracellular vesicles with the plasma membrane in order to release their contents. Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are involved in the fusion of secretory vesicles with the plasma membrane (reviewed in [1]). The exocyst complex is thought to mediate the tethering of vesicles to the membrane prior to fusion and is a multimeric protein complex highly conserved from yeast to mammals [2]. It was first identified in the budding yeast *Saccharomyces cerevisiae*, where it plays an essential role in exocytosis. The exocyst is composed of eight proteins: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 [2].

In yeast it has been shown that Sec3 [3] and Exo70 are localized at the plasma membrane [4], whereas the other subunits are localized at the membrane of the secretory vesicles [4] (Figure 1A). Recent studies have shown that Sec3 and Exo70 are recruited to the plasma membrane by interacting with phosphatidylinositol 4,5-biphosphate ($PI(4,5)P_2$) [5,6]. In particular, the carboxy-terminal domain of Exo70 (domain D) contains a succession of basic residues that are essential for the recruitment of the exocyst to the plasma membrane (Figure 1A). Mutations of those residues lead to mislocalization of the exocyst and a severe defect in secretion [5]. Similarly, the amino-terminal domain of Sec3 contains a cluster of basic residues that are responsible for the interaction with $PI(4,5)P_2$ [6].

The localization and function of the exocyst is tightly regulated by interactions with several small GTPases from the Rab, Rho, Arf and Ras families (for extensive review, see [7]). Of particular interest is a member of the Ras family, the small GTPase RalA. Yeast two-hybrid screen and pulldown assays showed that Sec5 interacts with the GTP-bound form of RalA [8]. The first functional studies on RalA showed that inhibition of RalA expression disturbs assembly of the exocyst complex, leads to a mislocalization of basolateral membrane proteins and impairs delivery of secretory vesicles to the plasma membrane [8]. Since then, numerous studies have reported a critical involvement of RalA not only in secretion but also in membrane trafficking [9]. In a recent issue of *Current Biology*, Nichols and Casanova [10] describe a new role for the exocyst and RalA as targets of the bacteria *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium).

S. Typhimurium is a Gram-negative bacterial pathogen and a significant cause of food poisoning and gastrointestinal inflammation. Upon interaction with the plasma membrane of the host cell, *S.* Typhimurium activates the expression of a type three secretion system (T3SS) encoded by the *Salmonella* pathogenicity island 1 (SPI-1). The T3SS is a needle-like structure complex, which allows the bacteria to translocate bacterial proteins (called effectors) directly into the host cytoplasm. SPI-1 effectors are involved in actin rearrangements and formation of membrane ruffles that facilitate the internalization of the bacteria [11]. Actin rearrangements

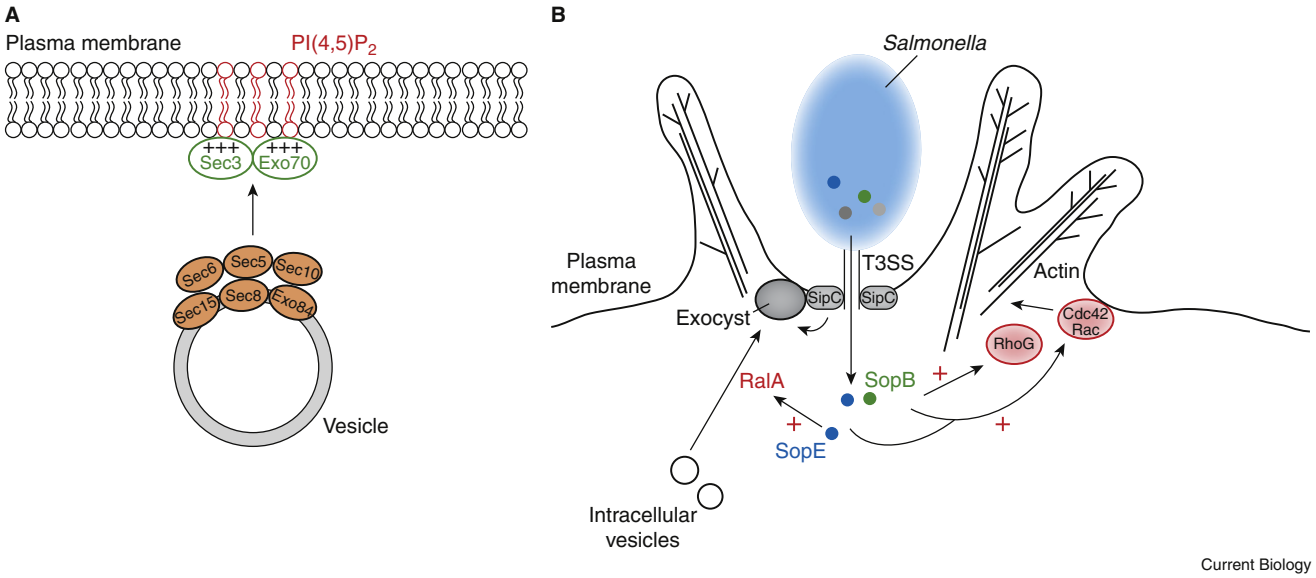

Figure 1. The exocyst during *Salmonella* invasion.

(A) Organization of the exocyst complex in *Saccharomyces cerevisiae*. The subunits Sec3 and Exo70 contain a group of positive residues that allow for an interaction with $PI(4,5)P_2$ and anchoring to the plasma membrane. (B) The exocyst complex is recruited to the *Salmonella* invasion site through interaction between the *Salmonella* effector SipC and the exocyst subunit Exo70. The *Salmonella* effector SopE activates the small GTPase RalA, which triggers the activation of the exocyst complex and results in the delivery of additional membrane to the site of invasion. SopE and the *Salmonella* effector SopB are also involved in the activation of RhoG, Cdc42 and Rac, leading to actin polymerization, which is required for entry of the bacteria.

induced by *S.* Typhimurium are initiated by the activation of Rho family GTPases. The *Salmonella* effectors SopE and SopE2 have been shown to act as guanine nucleotide exchange factors (GEFs) for Cdc42 and Rac, whereas the effector SopB is involved in the activation of Cdc42 and RhoG [11]. The activation of these GTPases then leads to actin polymerization (Figure 1B).

The effector SipC is a major component of the T3SS translocation system and is also involved in actin polymerization [11]. It has been recently shown that the carboxy-terminal domain of SipC binds to actin and induces the formation of actin bundles [12]. This bundling activity is essential for *Salmonella* invasion as *S.* Typhimurium mutants lacking this activity are less invasive. In their new work, Nichols and Casanova [10] demonstrate by two-hybrid analysis that the exocyst protein Exo70 is a binding partner of SipC. Pulldown assays and *in vitro* experiments confirm a direct interaction between the carboxy-terminal domain of SipC and Exo70. Additionally, the authors show that the exocyst components Sec5, Sec8, Sec10 and Exo70 are enriched at sites of bacterial attachment, suggesting that exocytic vesicles are targeted to sites of *Salmonella* invasion.

The authors also demonstrate that *Salmonella* invasion induces the activation of RalA, whereas a *S.* Typhimurium strain lacking SopE (Δ*sopE*) does not, indicating that SopE is required for RalA activation. The exocyst is also essential for *Salmonella* invasion because inhibition of the exocyst function, using small interfering RNA (siRNA) against Sec5, Exo70, and RalA, impairs bacterial internalization. Moreover, using quantitative microscopy measurements, the authors show that depletion of Sec5 and RalA by siRNA leads to a reduction of the size of *Salmonella* invasion foci. From these results, the authors propose a role for the exocyst in the delivery of vesicles to the site of bacterial entry to provide additional membrane to allow the extension and ruffling of the plasma membrane necessary to promote invasion.

Localized membrane delivery has been described during phagocytosis to accommodate the extension of the plasma membrane concomitant with the internalization of the particle [13]. In particular, Sec10 and Sec15 from the exocyst have been shown to interact at the site of phagocytosis with Arf6 [14], which plays a role in the regulation of membrane delivery to the phagocytic cup [15]. Moreover, SNAREs, such as vesicle-associated membrane proteins 3 and 7 (VAMP3 and VAMP7), have been implicated in the delivery of internal membrane pools coming from recycling endosomes [16] and late endosomes/lysosomes, respectively [17]. Since formation of the phagosome and formation of the *Salmonella*-containing vacuole (SCV) have some similarities [18], one could wonder whether *Salmonella* could recruit and target the same SNARE machinery via SPI-1 effectors to promote bacterial entry.

It has been shown that cytokines are released during phagosome formation and that, in particular, the export of tumor necrosis factor α to the plasma membrane at sites of phagocytic cup formation is dependent on VAMP3 [19] and the adaptor protein AP-1 [20]. It might therefore be possible that similar secretion occurs during *Salmonella* invasion at the site of membrane ruffling. Further studies need to be carried out to obtain a full picture of the host cell machinery involved in the fusion of intracellular vesicles at the *Salmonella* invasion foci and to understand how *Salmonella* modulates host proteins to promote invasion.

References

1. Chen, Y.A., and Scheller, R.H. (2001). SNARE-mediated membrane fusion. Nat. Rev. Mol. Cell Biol. *2*, 98–106.
2. TerBush, D.R., Maurice, T., Roth, D., and Novick, P. (1996). The exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. EMBO J. *15*, 6483–6494.
3. Finger, F.P., Hughes, T.E., and Novick, P. (1998). Sec3p is a spatial landmark for polarized secretion in budding yeast. Cell *92*, 559–571.
4. Boyd, C., Hughes, T., Pypaert, M., and Novick, P. (2004). Vesicles carry most exocyst subunits to exocytic sites marked by the remaining two subunits, Sec3p and Exo70p. J. Cell Biol. *167*, 889–901.
5. Liu, J., Zuo, X., Yue, P., and Guo, W. (2007). Phosphatidylinositol 4,5-bisphosphate mediates the targeting of the exocyst to the plasma membrane for exocytosis in mammalian cells. Mol. Biol. Cell *18*, 4483–4492.
6. Zhang, X., Orlando, K., He, B., Xi, F., Zhang, J., Zajac, A., and Guo, W. (2008). Membrane association and functional regulation of Sec3 by phospholipids and Cdc42. J. Cell Biol. *180*, 145–158.
7. Wu, H., Rossi, G., and Brennwald, P. (2008). The ghost in the machine: small GTPases as spatial regulators of exocytosis. Trends Cell Biol. *18*, 397–404.
8. Moskalenko, S., Henry, D.O., Rosse, C., Mirey, G., Camonis, J.H., and White, M.A. (2002). The exocyst is a Ral effector complex. Nat. Cell Biol. *4*, 66–72.
9. van Dam, E.M., and Robinson, P.J. (2006). Ral: mediator of membrane trafficking. Int. J. Biochem. Cell Biol. *38*, 1841–1847.
10. Nichols, C.D., and Casanova, J.E. (2010). *Salmonella*-directed recruitment of new membrane to invasion foci via the host exocyst complex. Curr. Biol. *20*, 1316–1320.
11. Zhou, D., and Galan, J. (2001). Salmonella entry into host cells: the work in concert of type III secreted effector proteins. Microbes Infect. *3*, 1293–1298.
12. Myeni, S.K., and Zhou, D. (2010). The C terminus of SipC binds and bundles F-actin to promote Salmonella invasion. J. Biol. Chem. *285*, 13357–13363.
13. Booth, J.W., Trimble, W.S., and Grinstein, S. (2001). Membrane dynamics in phagocytosis. Semin. Immunol. *13*, 357–364.
14. Stuart, L.M., Boulais, J., Charriere, G.M., Hennessy, E.J., Brunet, S., Jutras, I., Goyette, G., Rondeau, C., Letarte, S., Huang, H., *et al.* (2007). A systems biology analysis of the Drosophila phagosome. Nature *445*, 95–101.
15. Niedergang, F., Colucci-Guyon, E., Dubois, T., Raposo, G., and Chavrier, P. (2003). ADP ribosylation factor 6 is activated and controls membrane delivery during phagocytosis in macrophages. J. Cell Biol. *161*, 1143–1150.
16. Bajno, L., Peng, X.R., Schreiber, A.D., Moore, H.P., Trimble, W.S., and Grinstein, S. (2000). Focal exocytosis of VAMP3-containing vesicles at sites of phagosome formation. J. Cell Biol. *149*, 697–706.
17. Braun, V., Fraisier, V., Raposo, G., Hurbain, I., Sibarita, J.B., Chavrier, P., Galli, T., and Niedergang, F. (2004). TI-VAMP/VAMP7 is required for optimal phagocytosis of opsonised particles in macrophages. EMBO J. *23*, 4166–4176.
18. Brumell, J.H., and Grinstein, S. (2004). Salmonella redirects phagosomal maturation. Curr. Opin. Microbiol. *7*, 78–84.
19. Murray, R.Z., Kay, J.G., Sangermani, D.G., and Stow, J.L. (2005). A role for the phagosome in cytokine secretion. Science *310*, 1492–1495.
20. Braun, V., Deschamps, C., Raposo, G., Benaroch, P., Benmerah, A., Chavrier, P., and Niedergang, F. (2007). AP-1 and ARF1 control endosomal dynamics at sites of FcR mediated phagocytosis. Mol. Biol. Cell *18*, 4921–4931.

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Neurophysiology: Recording from Neurons in Action

Sensory neurons have mostly been studied in fixed animals, but how do they behave when the animal is free to move? A recent study shows that, during locomotor activity, besides there being a general enhancement in responsiveness, the tuning curves of neurons can also change, altering their optimal stimuli.

Alexander Borst

Much of what we know about nerve cells comes from experiments where the brain has been isolated from the animal, cut into slices and bathed in an artificial recording solution. The reason for that is that, in order to place a patch-electrode onto a neuron, visibility as well as stability are badly needed. For the study of sensory neurons, it is of course advisable to leave the brain attached to the sensory organs. So the next best situation is to anesthetize or at least to fix the animal, place them in front of a stimulus monitor or loudspeaker, play the stimulus and record from the neuron of interest. This has been the prevailing paradigm for decades, and most of what we read in textbooks about receptive fields and preferred orientation of visual interneurons or spectro-temporal filter properties of auditory neurons comes from such experiments. For a functional interpretation of these data in terms of behavioral relevance, the classical neuro-ethological approach has been to correlate a given behavior observed in mostly unrestrained animals with the response properties of neurons obtained from tethered animals. Thus, it was tacitly assumed that the neural response properties are the same, when the animal is immobilized passively perceiving the stimulus and when it is freely moving around. As is demonstrated by the recent work of Chiappe *et al.* [1,2], however, this assumption can no longer be made.

As they report in this issue of *Current Biology*, Chiappe *et al.* [1] investigated an identified visual, motion-sensitive interneuron in the brain of the fruit fly *Drosophila*, the so-called HSN-cell (*H*orizontal *S*ystem, *N*orthern). From previous work on immobilized flies, this cell was known to respond to large-field visual motion stimuli, such as vertical gratings moving from the front to the back of the fly [3]. In their new setting, instead of waxing the fly down on a platform to get stable access to its brain, the authors built on a technique that was invented more than 30 years ago for precise measurements of visually driven behavior [4]. They placed the fly on a little walking sphere made from polyurethane foam floating on an air cushion. With the fly held in place from the top, it is free to walk stationary on the sphere, the movement of which is conveniently detected by a camera system based on an optical computer mouse device allowing for a reconstruction of the path the fly would have taken if it was free to move.

To record the HSN-cell's activity optically, Chiappe *et al.* [1] expressed a recently developed genetically-encoded calcium indicator called GCaMP3.0 [5]. This indicator is based on the well-known green fluorescent protein (GFP), engineered to change its fluorescence along with changes of intracellular calcium